exposing the biological specimen to a probe that hybridizes to a human-*P. carinii* nucleic acid sequence, if the sequence is present in the sample to form a hybridization complex; and

determining whether the hybridization complex is present

wherein the nucleic acid sequence derived from human-*P. carinii* is an MSG encoding sequence.

24. (reiterated) The method of claim 23, wherein the labeled probe comprises a nucleic acid sequence according to SEQ ID NO: 19.

Please cancel claims 25-45.

Remarks

Claims 1-45 were pending in this application. Claims 25-45 are cancelled herein as drawn to non-elected Groups. Applicants expressly reserve the right to pursue protection of any or all of the cancelled claims in a subsequent application.

In addition, five paragraphs of the specification and claims 3, 4, 5, 6, and 7 are amended. Support for the amendments to replace "3132" with "3054" in the specification and in claims 4, 5, 6, and 7 can be found at least in the sequence listing, at page 43. Support for the amended language in claim 3, regarding 79% sequence identity, can be found in a direct comparison of the highly conserved regions of the disclosed human-*P. carinii* MSG-encoding sequences, as discussed more fully below. Support for the term "91%" in claim 5 can be found at least in the specification at page 9, line 10.

No new matter is introduced by these amendments. After entry of this amendment, **claims 1-24 are pending in the application.**

Correction of a Clerical Error

During preparation of this response, Applicants became aware of a clerical error that had been carried throughout the application, related to the length of nucleotide sequence of SEQ ID NO: ~~13~~. By this amendment, five paragraphs in the specification have been amended to correct this obvious error; claims 4, 5, 6, and 7 have been amended to correct the same error. Support for these amendments can be found on page 43 of the sequence listing as originally filed, which clearly shows that SEQ ID NO: ~~13~~ is 3054 residues in length, rather than 3132.

Restriction Between Groups I through IV

The Restriction Requirement contends that there are four inventions encompassed in the current application. Of the four Groups, Applicants elect to pursue the claims in Examiner's Group I in the current application. This group is directed to methods of detecting the presence of *Pneumocystis carinii* in a sample by amplifying a conserved region within a gene encoding a human *P. carinii* MSG protein.

Election of a Sequence Species

The Restriction Requirement contends that, within Group I a further restriction is required to elect a single sequence (inclusive of subsequences thereof) specified in the claims of this group. These claims refer to highly conserved regions of each of SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, and 15 that encode highly conserved regions of the corresponding proteins.

Applicants traverse the requirement to elect a single sequence, as making such an election would render aspects of the elected invention inoperative. At least certain of the claimed methods are intended to amplify portions of two or more of the provided human-*P. carinii* MSG-encoding sequences if they are present in the same sample. By requiring that the Applicants elect only one sequence, the Examiner would effectively eviscerate those embodiments of the invention that are intended to detect more than one sequence concurrently.

In addition, Applicants assert that there will be no serious burden on the Examiner to retain all of the specified highly conserved regions within the elected Group. In particular, Applicants point out that each of the specified sequences [particularly, residues 2894-3042 of

HMSGp1 (SEQ ID NO: 1), 2758-3006 of *HMSGp3* (SEQ ID NO: 3), 2845-3090 of *HMSG11* (SEQ ID NO: 5), 2839-3084 of *HMSG14* (SEQ ID NO: 7), 2836-3081 of *HMSG32* (SEQ ID NO: 9), 2887-3054 of *HMSG33* (SEQ ID NO: 11), 2821-3072 of *HMSG35* (SEQ ID NO: 13), and 1-249 of *HMSGp2* (SEQ ID NO: 15)] are substantially similar to one another, by definition.

To illustrate the inter-relatedness of these highly-conserved regions, Applicants provide in Table 1 (below) a summary of the percentages sequence identity that exists between these regions. Each of the specified regions shares at least 79% nucleic acid sequence identity with every other member of this defined group. If the Examiner searches based on the specified portion of SEQ ID NO: 13 for all sequences having 79% or greater identity, all of the specified highly conserved portions of SEQ ID NO: 1, 3, 5, 7, 9, 11, and 15 will also be searched. Therefore, there is no serious burden on the Examiner to maintain all of the sequences in the elected Group.

Table 1

Conserved region from	**SEQ 1**	**SEQ 3**	**SEQ 5**	**SEQ 7**	**SEQ 9**	**SEQ 11**	**SEQ 13**	**SEQ 15**
SEQ 1	**100**							
SEQ 3	93	**100**						
SEQ 5	82	81	**100**					
SEQ 7	79	79	94	**100**				
SEQ 9	83	79	93	91	**100**			
SEQ 11	79	79	93	100	92	**100**		
SEQ 13	88	84	94	94	89	91	**100**	
SEQ 15	97	89	79	80	79	82	85	**100**

In a further effort to retain all of the sequences in the elected claim Group, Applicants have amended claim 3 to include the phrase "wherein the highly conserved region has at least 79% sequence identity with residues 2821-3072 of *HMSG35* (SEQ ID NO: 13)." Support for this amended language can be found in a direct comparison of the conserved regions of the human-*P. carinii* MSG-encoding sequences, as illustrated in Table 1. As stated above, searching the databases for references that would be relevant to this claim will inherently identify references that would be relevant to the claims that include SEQ ID NO: 1, 3, 5, 7, 9, 11, and 15, as well as SEQ ID NO: 13. Therefore, Applicants request that the requirement for an election of

a single sequence species be withdrawn. If the Examiner refuses to withdraw this requirement, Applicants provisionally elect SEQ ID NO: 13.

Conclusion

In light of the above arguments and amendments, and for the reasons stated above, Applicants request that the Restriction Requirement be modified. Examiner Goldberg is invited to telephone the undersigned if any questions remain concerning the requirement for restriction. Otherwise, the present application is ready for substantive examination, and such action is requested.

Respectfully submitted,

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Marked-up Version of Amended Specification and Claims
Pursuant to 37 C.F.R. §§ 1.121(b)-(c)

In the Specification:

At page 2, line 37 through page 3, line 13:

This invention encompasses the purified novel human-*P. carinii* proteins represented by SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, and SEQ ID NO: 14, and isolated nucleic acid molecules that encode these proteins. Specific nucleic acid molecules encompassed in this invention include those represented in SEQ ID NO: 1; SEQ ID NO: 2; SEQ ID NO: 3; SEQ ID NO: 4, SEQ ID NO: 5; SEQ ID NO: 6, SEQ ID NO: 7; SEQ ID NO: 15; and SEQ ID NO: 17. Also encompassed within this invention are the isolated nucleic acid sequences that encode the carboxy-terminal conserved about 100 amino acids of the disclosed human-*P. carinii MSG*s; these may be used for amplification or as probes. The sequences of these conserved nucleic acid molecule regions include residues 2894-3042 of *HMSGp1* (SEQ ID NO: 1), 2758-3006 of *HMSGp3* (SEQ ID NO: 3), 2845-3090 of *HMSG11* (SEQ ID NO: 5), 2839-3084 of *HMSG14* (SEQ ID NO: 7), 2836-3081 of *HMSG32* (SEQ ID NO: 9), 2887-3054~~3132~~ of *HMSG33* (SEQ ID NO: 11), 2821-3072 of *HMSG35* (SEQ ID NO: 13), or 1-249 of *HMSGp2* (SEQ ID NO: 15). In addition, this invention encompasses sequences with at least 70% sequence identity to these regions, and recombinant vectors comprising such nucleic acid molecules and conserved regions from within such nucleic acid molecules, as well as transgenic cells including such a recombinant vector.

At page 3, lines 14-31:

Another aspect of this invention provides a method of detecting the presence of *Pneumocystis carinii* in a biological specimen, by amplifying with a nucleic acid amplification method (*e.g.*, the polymerase chain reaction) a human-*P. carinii* nucleic acid sequence using two or more oligonucleotide primers derived from a human-*P. carinii* MSG protein encoding sequence, then determining whether an amplified sequence is present. In a preferred embodiment of this invention, the human-*P. carinii* nucleic acid sequence is a highly conserved region within an MSG-protein encoding sequence. Such a highly conserved region may, for

instance, include residues 2894-3042 of *HMSGp1* (SEQ ID NO: 1), 2758-3006 of *HMSGp3* (SEQ ID NO: 3), 2845-3090 of *HMSG11* (SEQ ID NO: 5), 2839-3084 of *HMSG14* (SEQ ID NO: 7), 2836-3081 of *HMSG32* (SEQ ID NO: 9), 2887-3054~~3132~~ of *HMSG33* (SEQ ID NO: 11), 2821-3072 of *HMSG35* (SEQ ID NO: 13), or 1-249 of *HMSGp2* (SEQ ID NO: 15). A further aspect of this invention is the method of detecting the presence of *Pneumocystis carinii* in a biological specimen, by determining whether an amplified sequence is present, for instance by electrophoresis and staining of the amplified sequence, or hybridization to a labeled probe of the amplified sequence. Appropriate labels for the hybridization probe include a fluorescent molecule, a chemiluminescent molecule, an enzyme, a co-factor, an enzyme substrate, or a hapten. The nucleotide sequence of such a probe can be chosen from any *MSG* gene sequence that is amplified in the detection method, and for instance can include a nucleic acid sequence according to SEQ ID NO: 19.

At page 8, lines 28-33:

Further nucleic acid molecules might comprise at least 15 consecutive nucleotides of the regions encoding the conserved carboxy-terminal portion of each human-*P. carinii MSG* gene. These regions comprise nucleotides 2894-3042 of *HMSGp1* (SEQ ID NO: 1), 2758-3006 of *HMSGp3* (SEQ ID NO: 3), 2845-3090 of *HMSG11* (SEQ ID NO: 5), 2839-3084 of *HMSG14* (SEQ ID NO: 7), 2836-3081 of *HMSG32* (SEQ ID NO: 9), 2887-3054~~3132~~ of *HMSG33* (SEQ ID NO: 11), 2821-3072 of *HMSG35* (SEQ ID NO: 13), and 1-249 of *HMSGp2* (SEQ ID NO: 15), respectively.

At page 12, line 35, through page 13, line 13:

Oligonucleotides that are derived from the human-*P. carinii HMSGp1*, *HMSGp3*, *HMSG11*, *HMSG14*, *HMSG32*, *HMSG33*, and *HMSG35* gene sequences (SEQ ID NOS: 1, 3, 5, 7, 9, 11, and 13, respectively), as well as the fragment of *HMSGp2* disclosed (SEQ ID NO: 15), are encompassed within the scope of the present invention. Preferably, such oligonucleotide primers will comprise a sequence of at least 15-20 consecutive nucleotides of the relevant human-*P. carinii MSG* gene sequence. To enhance amplification specificity, oligonucleotide primers comprising at least 25, 30, 35, 40, 45 or 50 consecutive nucleotides of these sequences may also be used. These primers for instance may be obtained from any region of the disclosed

sequences. By way of example, human-*P. carinii MSG* gene sequences may be apportioned into halves or quarters based on sequence length, and the isolated nucleic acid molecules may be derived from the first or second halves of the molecules, or any of the four quarters. In addition, primers may be specifically chosen from the conserved carboxy-terminal region of each *MSG* coding sequence. This region comprises nucleic acid residues 2894-3042 of *HMSGp1* (SEQ ID NO: 1), 2758-3006 of *HMSGp3* (SEQ ID NO: 3), 2845-3090 of *HMSG11* (SEQ ID NO: 5), 2839-3084 of *HMSG14* (SEQ ID NO: 7), 2836-3081 of *HMSG32* (SEQ ID NO: 9), 2887-3054~~3132~~ of *HMSG33* (SEQ ID NO: 11), 2821-3072 of *HMSG35* (SEQ ID NO: 13), and 1-249 of *HMSGp2* (SEQ ID NO: 15).

At page 17, line 34, though page 18, line 6:

The selection of PCR primers will be made according to the portions of the gene sequence that are to be amplified. For use in PCR detection of *P. carinii*, it is advantageous to choose primer-annealing sites that are highly conserved across many different members of the human-*P. carinii MSG* gene family. For instance, it is advantageous to choose primer sites from within the regions of human-*P. carinii* sequence displaying greater than 63% sequence identity across the disclosed family members, *e.g.*, that portion of the gene encoding the conserved carboxy-terminal region of the protein. The highly conserved carboxy-terminal regions of the disclosed genes are as follows: residues 2894-3042 of *HMSGp1* (SEQ ID NO: 1), 2758-3006 of *HMSGp3* (SEQ ID NO: 3), 2845-3090 of *HMSG11* (SEQ ID NO: 5), 2839-3084 of *HMSG14* (SEQ ID NO: 7), 2836-3081 of *HMSG32* (SEQ ID NO: 9), 2887-3054~~3132~~ of *HMSG33* (SEQ ID NO: 11), 2821-3072 of *HMSG35* (SEQ ID NO: 13), and 1-249 of *HMSGp2* (SEQ ID NO: 15).

In the Claims:

1. A method of detecting the presence of *Pneumocystis carinii* in a biological specimen, comprising:

amplifying a highly conserved region within a human-*P. carinii* nucleic acid sequence, if such sequence is present in the sample, using two or more oligonucleotide primers derived from human-*P. carinii* MSG protein encoding sequence; and

determining whether an amplified sequence is present.

2. The method according to claim 1, wherein amplification of the human-*P. carinii* nucleic acid sequence is by polymerase chain reaction.

3. The method of claim 1, wherein the human-*P. carinii* nucleic acid sequence is a highly conserved region within an MSG-protein encoding sequence, wherein the highly conserved region has at least 79% sequence identity with residues 2821-3072 of *HMSG35* (SEQ ID NO: 13).

4. The method of claim 3, wherein the highly conserved region comprises a sequence selected from the group consisting of: residues 2894-3042 of *HMSGp1* (SEQ ID NO: 1), 2758-3006 of *HMSGp3* (SEQ ID NO: 3), 2845-3090 of *HMSG11* (SEQ ID NO: 5), 2839-3084 of *HMSG14* (SEQ ID NO: 7), 2836-3081 of *HMSG32* (SEQ ID NO: 9), 2887-~~3132~~3054 of *HMSG33* (SEQ ID NO: 11), 2821-3072 of *HMSG35* (SEQ ID NO: 13), and 1-249 of *HMSGp2* (SEQ ID NO: 15).

5. The method of claim 1, wherein at least one oligonucleotide primer comprises at least 15 contiguous nucleotides from a sequence chosen from the group consisting of: residues 2894-3042 of *HMSGp1* (SEQ ID NO: 1), 2758-3006 of *HMSGp3* (SEQ ID NO: 3), 2845-3090 of *HMSG11* (SEQ ID NO: 5), 2839-3084 of *HMSG14* (SEQ ID NO: 7), 2836-3081 of *HMSG32* (SEQ ID NO: 9), 2887-3054~~3132~~ of *HMSG33* (SEQ ID NO: 11), 2821-3072 of *HMSG35* (SEQ ID NO: 13), and 1-249 of *HMSGp2* (SEQ ID NO: 15) and nucleic acid sequences having at least ~~70%~~91% sequence homology with residues 2894-3042 of *HMSGp1* (SEQ ID NO: 1), 2758-3006 of *HMSGp3* (SEQ ID NO: 3), 2845-3090 of *HMSG11* (SEQ ID NO: 5), 2839-3084 of *HMSG14* (SEQ ID NO: 7), 2836-3081 of *HMSG32* (SEQ ID NO: 9), 2887-3054~~3132~~ of *HMSG33* (SEQ ID NO: 11), 2821-3072 of *HMSG35* (SEQ ID NO: 13), and 1-249 of *HMSGp2* (SEQ ID NO: 15).

6. The method of claim 5, wherein at least one oligonucleotide primer comprises at least 15 contiguous nucleotides from a nucleic acid sequence having at least 90% sequence homology with residues 2894-3042 of *HMSGp1* (SEQ ID NO: 1), 2758-3006 of *HMSGp3* (SEQ ID NO: 3), 2845-3090 of *HMSG11* (SEQ ID NO: 5), 2839-3084 of *HMSG14* (SEQ ID NO: 7), 2836-3081 of *HMSG32* (SEQ ID NO: 9), 2887-3054~~3132~~ of *HMSG33* (SEQ ID NO: 11), 2821-3072 of *HMSG35* (SEQ ID NO: 13), and 1-249 of *HMSGp2* (SEQ ID NO: 15).

7. The method of claim 5, wherein at least one oligonucleotide primer comprises at least 15 contiguous nucleotides from a nucleic acid sequence having at least 95% sequence homology with residues 2894-3042 of *HMSGp1* (SEQ ID NO: 1), 2758-3006 of *HMSGp3* (SEQ ID NO: 3), 2845-3090 of *HMSG11* (SEQ ID NO: 5), 2839-3084 of *HMSG14* (SEQ ID NO: 7), 2836-3081 of *HMSG32* (SEQ ID NO: 9), 2887-3054~~3132~~ of *HMSG33* (SEQ ID NO: 11), 2821-3072 of *HMSG35* (SEQ ID NO: 13), and 1-249 of *HMSGp2* (SEQ ID NO: 15).

8. The method of claim 5, wherein the oligonucleotide primers are chosen from the group consisting of: SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 23, and SEQ ID NO: 24.

9. The method of claim 5, wherein the pair of oligonucleotide primers consist of one upstream primer and one downstream primer.

10. The method of claim 9, wherein:

the upstream primer is chosen from the group consisting of: SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 23; and

the downstream primer is chosen from the group consisting of: SEQ ID NO: 20 and SEQ ID NO: 24.

11. The method of claim 8, wherein one of the oligonucleotide primers comprises SEQ ID NO: 17.

12. The method of claim 8, wherein one of the oligonucleotide primers comprises SEQ ID NO: 18.

13. The method of claim 8, wherein one of the oligonucleotide primers comprises SEQ ID NO: 19.

14. The method of claim 8, wherein one of the oligonucleotide primers comprises SEQ ID NO: 20.

15. The method of claim 8, wherein one of the oligonucleotide primers comprises SEQ ID NO: 23.

16. The method of claim 8, wherein one of the oligonucleotide primers comprises SEQ ID NO: 24.

17. The method of claim 1, wherein the biological specimen is from the oropharyngeal tract.

18. The method of claim 1, wherein the biological specimen is from blood.

19. The method of claim 1, wherein the step of determining whether an amplified sequence is present comprises one or more of:

(a) electrophoresis and staining of the amplified sequence; or

(b) hybridization to a labeled probe of the amplified sequence.

20. The method of claim 19, wherein the amplified sequence is detected by hybridization to a labeled probe.

21. The method of claim 22, wherein the probe comprises a detectable non-isotopic label chosen from the group consisting of:

a fluorescent molecule;

a chemiluminescent molecule;

an enzyme;

a co-factor;

an enzyme substrate; and

a hapten.

22. The method of claim 21, wherein the labeled probe comprises a nucleic acid sequence according to SEQ ID NO: 19.

23. A method of detecting the presence of *Pneumocystis carinii* in a biological specimen, comprising:

exposing the biological specimen to a probe that hybridizes to a human-*P. carinii* nucleic acid sequence, if the sequence is present in the sample to form a hybridization complex; and

determining whether the hybridization complex is present

wherein the nucleic acid sequence derived from human-*P. carinii* is an MSG encoding sequence.

24. The method of claim 23, wherein the labeled probe comprises a nucleic acid sequence according to SEQ ID NO: 19.

25. through 45. (Cancel)